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# Prevalence of *Toxoplasma gondii* in cats from Colombia, South America and genetic characterization of *T. gondii* isolates

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**Abstract**

Cats are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can excrete the environmentally-resistant oocysts. In the present study, prevalence of *T. gondii* was determined in serum, feces, and tissues of 170 unwanted cats from Colombia, South America. Antibodies to *T. gondii* were assayed by the modified agglutination test and found in 77 of 170 (45.2%) cats with titers of <1:5 in 93, 1:5 in eight, 1:10 in 17, 1:20 in 10, 1:40 in seven, 1:80 in four, 1:160 in eight, 1:320 in six, and 1:640 or higher in 17 cats. *T. gondii* oocysts were not found in feces of any cat as ascertained by bioassay in mice. Tissues (brain, heart, tongue) of 116 cats were bioassayed in mice or cats. *T. gondii* was isolated from tissues of 15 of the 42 cats with titers of 1:40 or higher and not from any of the 90 cats titers of 1:20 or lower. Of the 29 cats whose tissues were bioassayed individually, *T. gondii* was isolated from the tongues of nine, hearts of eight, and brains of five. Mice inoculated with tissues of 12 of 15 infected cats died of toxoplasmosis; with nine *T. gondii* isolates all infected mice died. Overall, 65 of 92 (70%) of *T. gondii*-infected mice died of toxoplasmosis. Genotyping of these 15 isolates using polymorphisms at the SAG1, SAG2, SAG3, BTUB, and GRA6 loci revealed that three isolates (TgCtCo1, 2, and 7) had Type I alleles and one isolate (TgCtCo8) had Type II allele at all five loci. Eleven isolates contained the combination of Type I and III alleles and were divided into three genotypes, with TgCtCo3,5,6,9,12,13 and 15 had alleles I, I, III, I and III, TgCtCo4,10,11 had alleles I, III, III, I and I, and TgCtCo14 had alleles I, III, III, III, and III, at loci SAG1, SAG2, SAG3, BTUB and GRA6, respectively. All infected mice from

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each group had identical genotype except one mouse infected with TgCtCo5 had a Type III allele at locus BTUB and a unique allele (u-1) at locus SAG1 indicating mixed infection for TgCtCo5, whereas the rest seven mice had a Type I alleles at both loci. Published by Elsevier B.V.

*Keywords:* *Toxoplasma gondii*; Cats; Colombia; South America; Feces; Antibodies; Genotype

## 1. Introduction

*Toxoplasma gondii* infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988). Cats are important in the natural life cycle of *T. gondii* because they are the only hosts that can directly spread *T. gondii* in the environment. It is important to biologically and genetically characterize isolates of *T. gondii* from cats and this has been achieved only from cats from Brazil (Dubey et al., 2004; Pena et al., 2006). The objectives of the present study were to determine prevalence of *T. gondii* in cats from Colombia, South America and characterize isolates of *T. gondii* from these cats.

## 2. Materials and methods

In total, 170 (56 males, 85 females, for 29 sex not recorded) cats were obtained from Colombia, South America (Table 1). These cats were unwanted or stray and were euthanized by intravenous injection of Euthanex® (Invet S.A., Bogotá, Colombia) by Centro Distrital de Zoonosis as per the directorate from Secretariá de Salud de Bogotá and Armenia when efforts to place them in good homes failed. The cats were received in six batches (A–F) from May to November, 2005. From each cat, serum, brain, and heart, and feces were obtained for *T. gondii* examination. In addition, tongues were obtained from cats in batches C–F. Samples were transported refrigerated by air from Colombia to the Animal Parasitic Diseases Laboratory, United States Department of Agriculture (USDA) Beltsville, MD, where all *T. gondii* evaluations were performed using protocols approved by the USDA. Three to five days elapsed between euthanasia and examination for *T. gondii* and during this time samples were kept cold.

Sera from cats were diluted two-fold starting at 1:5 or 1:10 dilution and assayed for *T. gondii* antibodies with the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987).

Feces (2–10 g) collected from the rectum of 143 cats were floated in sugar solution and a drop from the meniscus was examined microscopically between cover slip and glass slide. Fecal floats were sedimented in water, and aerated in 2% sulfuric acid on a shaker at 22 °C for 1 week and then bioassayed in mice orally (Dubey and Beattie, 1988).

Tissues of 116 from 170 cats were bioassayed for *T. gondii*; tissues from 92 cats were bioassayed in mice

Table 1
Prevalence of *T. gondii* in cats from Colombia, South America

| Experiment number and batch | Date | Area | Number of cats | Number seronegative (<1:5) | Number seropositive (≥1:5) | Number bioassayed in mice | Number bioassayed in cats | *T. gondii* isolated |
|---|---|---|---|---|---|---|---|---|
| Tx 186 A | 05.26.05 | Armenia | 25 | 3 | 22 | 25 (0[a] + 25[b]) | 0 | 4 |
| TX 193 B | 08.05.05 | Armenia | 8 | 2 | 6 | 8 (4[a] + 4[c]) | 0 | 1 |
| TX 196 C | 08.24.05 | Bogota | 42 | 25 | 17 | 17 (10[a] + 7[c]) | 0 | 7 |
| TX 196 D | 09.22.05 | Bogota | 16 | 13 | 3 | 16 (3[a] + 13[c]) | 0 | 0 |
| TX 204 E | 10.12.05 | Bogota | 43 | 26 | 17 | 14 (3[a] + 11[c]) | 0 | 2 |
| TX 210 F | 11.07.05 | Bogota | 36 | 24 | 12 | 12 (9[a] + 3[c]) | 24 | 2 |
| Total | | | 170 | 93 | 77 | 92 | 24 | 16 |

[a] Brain, heart, tongue of each cat were bioassayed individually.
[b] Brain and hearts of each cat were pooled.
[c] Brain, heart, tongue of each cat pooled.

and from 24 cats were bioassayed in cats. Hearts and brains from each cat (irrespective of serology) in batch A were pooled and bioassayed in mice (Dubey, 1998). For cats in batches B–F, tongue, brain, and heart of each of the 29 cats were bioassayed individually in mice and from the remaining 63 cats, tongue (25 g), brain (25 g), and heart (25 g) of each cat were pooled and bioassayed in mice. Additionally, tongues, brains, and hearts from 24 cats with MAT titers of <1:5 were pooled and fed to two *T. gondii*-free cats as described (Dubey et al., 2004). Feces of cats were examined for shedding of *T. gondii* oocysts 3–14 days after feeding feline tissues as previously described (Dubey et al., 2004). Fecal floats from these cats were bioassayed orally in mice (Dubey and Beattie, 1988).

For mouse bioassay, each tissue was homogenized in five volumes (w/v) of saline, mixed with five volumes of acidic pepsin and the mixture incubated in a shaker water bath for 1 h at 37 °C. The digest was centrifuged, neutralized, mixed with antibiotics, and the homogenate was inoculated subcutaneously in five mice (Dubey, 1998). The mice used were Swiss Webster albino females obtained from Taconic Farms, Germantown, New York. Mice were examined for viable *T. gondii* parasites as described (Dubey et al., 2004). Mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were demonstrable in their tissues.

*T. gondii* DNA was extracted from the tissues of all infected mice from each group and strain typing was performed using genetic markers SAG1, SAG2, SAG3, BTUB and GRA6 as described (see Dubey et al., in press).

## 3. Results

Antibodies to *T. gondii* were found in 77 of 170 cats but in 35 of these titers were <1:40 (Table 2).

*T. gondii* was isolated from tissues of 15 cats; from 14 of 17 cats with titers of 1:640 or higher and from 1 of 21 cats with titers of 1: 40–1:320 (Table 3). *T. gondii* tachyzoites or tissue cysts were found in tissues of all infected mice from these 15 isolates and all of these isolates were cryopreserved for future studies. Additionally, all five mice inoculated with tissues of the cat 22 in batch A developed MAT antibodies when bled day 44 p.i. but tissue cysts were not found in their brains after an intensive search when these mice were killed 48 day p.i. Brain homogenates from these mice were subinoculated in to five mice; these mice developed antibodies but *T. gondii* was not demonstrable in their brains.

Table 2
Seroprevalence of *T. gondii* antibodies in cats from Colombia, South America

| Batch Tx | Total | <5 | 5 | 10 | 20 | 40 | 80 | 160 | 320 | 640 |
|---|---|---|---|---|---|---|---|---|---|---|
| A-186 | 25 | 3 | 0 | 1 | 5 | 3 | 1 | 4 | 3 | 5 |
| B-193 | 8 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 2 |
| C-196 | 42 | 25 | 3 | 4 | 1 | 1 | 0 | 0 | 2 | 6 |
| D-196 | 16 | 13 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| E-204 | 43 | 26 | 3 | 9 | 2 | 1 | 0 | 0 | 0 | 2 |
| F-210 | 36 | 24 | 0 | 1 | 0 | 1 | 3 | 4 | 1 | 2 |
| Total | 170 | 93 | 8 | 17 | 10 | 7 | 4 | 8 | 6 | 17 |

Of the 29 seropositive cats (Table 1) whose tissues were bioassayed individually, *T. gondii* was isolated from the tongues of nine, hearts of eight, and brains of five (Table 3). Mice inoculated with tissues of 12 of 15 cats died of toxoplasmosis; with nine *T. gondii* isolates all infected mice died. Overall, 65 of 92 (70%) of *T. gondii*–infected mice died of toxoplasmosis, mostly from toxoplasmic pneumonia.

The two cats fed tissues of 24 seronegative cats did not shed oocysts as ascertained by bioassay of fecal floats.

*T. gondii* oocysts were not found (both microscopically and by bioassay) in feces of any of the naturally-exposed cats from Colombia.

Genotyping of these 15 isolates using polymorphisms at the SAG1, SAG2, SAG3, BTUB, and GRA6 loci revealed that three isolates (TgCtCo1, 2, and 7) had Type I alleles and one isolate (TgCtCo8) had Type II allele at all five loci. Eleven isolates contained the combination of Type I and III alleles and were divided into three genotypes, with TgCtCo3,5,6,9,12,13 and 15 had alleles I, I, III, I and III, TgCtCo4,10,11 had alleles I, III, III, I and I, and TgCtCo14 had alleles I, III, III, III, and III, at loci SAG1, SAG2, SAG3, BTUB and GRA6, respectively. All infected mice from each group had identical genotype except one mouse infected with TgCtCo5 had a Type III allele at locus BTUB and a unique allele (u-1) at locus SAG1, whereas the rest seven mice had a Type I alleles at both loci. This indicates a mixed infection in TgCtCo5.

Table 3
Isolation of *T. gondii* from tissues of cats from Colombia, South America

| Cat number and batch | MAT | *T. gondii* isolation in mice[a] | | | | | | | | | Strain designation | PCR-RFLP genotype | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brain | | | Heart | | | Tongue | | | | SAG1 | SAG2 | SAG3 | BTUB | GRA6 |
| | | Number infected | Number died | Days of death | Number infected | Number died | Days of death | Number infected | Number died | Days of death | | | | | | |
| (Tx 186) | | | | | | | | | | | | | | | | |
| 8 A | ≥640 | 5 | 5 | 12,12,12,12,12 | Pooled with brain | | | ND[b] | | | TgCtCo 1 | I (5)[c] | I (5) | I (5) | I (5) | I (5) |
| 23 A | ≥640 | 4 | 3 | 16,17,24 | Pooled with brain | | | ND | | | TgCtCo 2 | I (3) | I (3) | I (3) | I (3) | I (3) |
| 24 A | ≥640 | 4 | 0 | | Pooled with brain | | | ND | | | TgCtCo 3 | I (4) | I (4) | III (4) | I (4) | III (4) |
| (Tx 193) | | | | | | | | | | | | | | | | |
| 6 B | ≥640 | 0 | | | 5 | 5 | 12,13,13,15,15 | 0 | | | TgCtCo 4 | I (5) | III (5) | III (5) | I (5) | I (5) |
| (Tx 196) | | | | | | | | | | | | | | | | |
| 3 C | ≥640 | 4 | 3 | 19,26,28 | 0 | | | 5 | 5 | 17,20,22,28,31 | TgCtCo 5 | u-1 (1) I (7) | I (8) | III (8) | I (7) III (1) | III (8) |
| 6 C | ≥640 | 1 | 1 | 22 | 1 | 1 | 22 | 4 | 4 | 18,22,24,28 | TgCtCo 6 | I (8) | I (8) | III (8) | I (8) | III (8) |
| 7 C | ≥640 | 1 | 1 | 22 | 0 | | | 1 | 1 | 12 | TgCtCo 7 | I (1) | I (1) | I (1) | I (1) | I (1) |
| 22 C | ≥640 | 5 | 0 | | 0 | | | 4 | 0 | | TgCtCo 8 | II or III (9) | II (9) | II (9) | II (9) | II (9) |
| 26 C | ≥640 | 0 | | | 5 | 2 | 13,19 | 5 | 5 | 9,11,21,21,24 | TgCtCo 9 | I (9) | I (9) | III (9) | I (9) | III (9) |
| 33 C | 320 | 0 | | | 2 | 0 | | 5 | 5 | 11,11,12,12,12 | TgCtCo 10 | I (7) | III (7) | III (7) | I (7) | I (7) |
| 34 C | 40 | 0 | | | 5 | 5 | 12,15,15,17,17 | 4 | 4 | 12,12,12,13 | TgCtCo 11 | I (9) | III (9) | III (9) | I (9) | I (9) |
| (Tx 204) | | | | | | | | | | | | | | | | |
| 39 E | ≥640 | 5 | 3 | 14,15,15 | 4 | 0 | | 5 | 3 | 14,15,18 | TgCtCo 12 | I (14) | I (14) | III (14) | I (14) | III (14) |
| 40 E | ≥640 | 5 | 0 | | 1 | 0 | | 0 | | | TgCtCo 13 | I (6) | I (6) | III (6) | I (6) | III (6) |
| (Tx 210) | | | | | | | | | | | | | | | | |
| 8 F | ≥640 | 0 | | | 0 | | | 5 | 5 | 11,14,14,14,15 | TgCtCo 14 | I (5) | III (5) | III (5) | III (5) | III (5) |
| 34 F | ≥640 | 0 | | | 2 | 2 | 16,18 | 0 | | | TgCtCo 15 | I (2) | I (2) | III (2) | I (2) | III (2) |

[a] Five mice were inoculated with each tissue.
[b] ND = Not done.
[c] Number in parenthesis are the number of mice used separately for genotyping.

## 4. Discussion

Until recently, little was known of the tissue distribution of *T. gondii* in cats. *T. gondii* is considered to have an affinity for encystment in neural tissue but this assumption is based on infections in mice. Results of the present study indicate that the cat tongue is more heavily parastized with *T. gondii* than the brain and supports earlier findings that muscles are parasitized more than the brain in cats (Dubey, 1977, 1997; Dubey et al., 2004).

Failure to find *T. gondii* oocysts in feces from any of the 143 cats in the present study is surprising and unexplained. Epidemiological data indicate that in any given time approximately 1% of cats are likely to have oocysts in their feces (Dubey and Beattie, 1988). Pena et al. (2006) found *T. gondii* oocysts in three of 237 cats from São Paulo, Brazil.

In the present study, antibodies to *T. gondii* were found in 45.2% of 170 cats tested by the MAT and all titers were recorded because the MAT titer that should be considered diagnostic in cats has not been determined. In other hosts, we have considered a MAT titer of 1:20 as specific for *T.* gondii infection. If we disregard titers of 1:10 or less as non-specific then the seroprevalence becomes 32.3% (52 of 170 cats). Little is known of the prevalence of *T. gondii* in cats from Colombia. Jewell et al. (1973) found dye test antibodies in titers of 1:8 or higher in 112 of 181 (62%) in cats from Medellin, Colombia. Montoya-Londoño et al. (1998) reported indirect florescent antibody titers in 25 of 28 (89.3%) cats from the city of Armenia; this seroprevalence is similar to 84% (28 of 33) seropositivity from the same city in the present study but markedly different from 35% (49 of 137) seroprevalence in cats from Bogota. Montoya-Londoño et al. (1998) also reported *T. gondii*- like oocysts in 66.6% of 28 cats from Armenia, Colombia but provided no details to judge their results.

In the present study of *T. gondii* isolates, results of cats from Colombia were similar to those obtained from chickens from Colombia (Dubey et al., 2005), except that one of the 14 isolates was Type II. Recently, Gallego et al. (2006) characterized *T. gondii* DNA obtained directly from infected tissues of 33 donor hosts (12 human, 2 birds, brains and hearts of 8 cats, 1 from cat feces, 1 opossum, 1 guinea pig) and found that at the SAG2 locus 31 specimens were Type I, one was Type III and one was atypical. Unlike this report, genetic characterization in the present study was based on DNA from viable *T. gondii* isolates. Overall, the rarity of Type II strain in Colombia and in Brazil (Dubey et al., 2004; Pena et al., 2006) further support the notion that the population structure is different in South America from that of North America and Europe (Lehmann et al., 2004).

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